UCRL-JC-123991 ABS

CYTOGENETIC AND DEVELOPMENTAL DEFECTS IN MOUSE ZYGOTES AND PREIMPLANTATION EMBRYOS AFTER PATERNAL EXPOSURE TO ACRYLAMIDE

F. Marchetti[a], T.E. Ahlborn[a,b], N. Titenko-Holland[b], X. Lowe[a], M.T. Smith[b], A.J. Wyrobek[a]

aBiology and Biotechnology Research Program, Lawrence Livermore National Laboratory, 7000 East Ave, Livermore 94550, CA, USA; bSchool of Public Health, University of California, 140 Warren Hall, Berkeley 94720, CA, USA

Genetic defects transmitted via sperm can lead to chromosomal aberrations detected in zygotes but little is known about the developmental consequences for preimplantation embryos. Male mice treated with the model clastogen acrylamide (AA, 50 mg/kg/day x 5 d) were mated with untreated females at various time intervals after treatment to investigate the effects on specific cell types of spermatogenesis. Over 3000 zygotes collected 18 hr after fertilization were charecterized cytologically and cytogenetically. Eggs that did not reach first cleavage metaphase were classified as unfertilized or developmentally delayed. Chromosome structural aberrations in zygote metaphases were evaluated using a combined DAPI and FISH painting analysis with four biotin-labeled probes specific for chromosomes 1, 2, 3, and X, plus a digoxigenin-labeled probe specific for chromosome Y. Unfertilized eggs, developmentally delayed and zygotes with chromosome structural aberrations were considered abnormal zygotes. Over 2000 4-day embryos, flushed from the uterine horns were evaluated under a stereo microscope and assigned to various categories of abnormalities: 1) arrested at one cell, 2) delayed and 3) with lysed blastomeres . After postmeiotic treatment, a good correlation between percentages of abnormal zygotes and abnormal 4-day embryos was observed for sperm treatment (84% vs 93%), however, there were more abnormal 4-day embryos than zygotes ($P<0.001$) after treatment of late spermatids (64% vs 85%) and early spermatids (27% vs 82%). While similar percentages of abnormal zygotes and 4-day embryos were produced in matings after treatment of spermatocytes (31% vs 31%), stem cells treatment also produced an excess of abnormal 4-day embryos (21% vs 49%). The discrepancies between percentages of abnormal zygotes and abnormal 4-day embryos observed after treatment of spermatids and stem cells may be due to AA-induced gene mutations, small deletions or epigenetic changes that affected embryo development but were not detected by cytogenetic analysis of zygotes. These findings suggest that the combined cytological and cytogenetic analyses of zygotes and 4-day embryos is a promising approach to characterize the susceptibility of male germ cells to environmental insults and to evaluate the developmental consequences of genetic damage transmitted via paternal germ cells.

[Work was performed under the auspices of the U.S. DOE by the Lawrence Livermore National Laboratory under contract W-7405-ENG-48 with support from NIEHS Y01-ES-10203-00 and Center for Molecular Cytology, UC San Francisco].